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Use of PBPK modelling to evaluate the performance of Dissolv*It*, a biorelevant dissolution assay for orally inhaled drug products

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MOLECULAR PHARMACEUTICS

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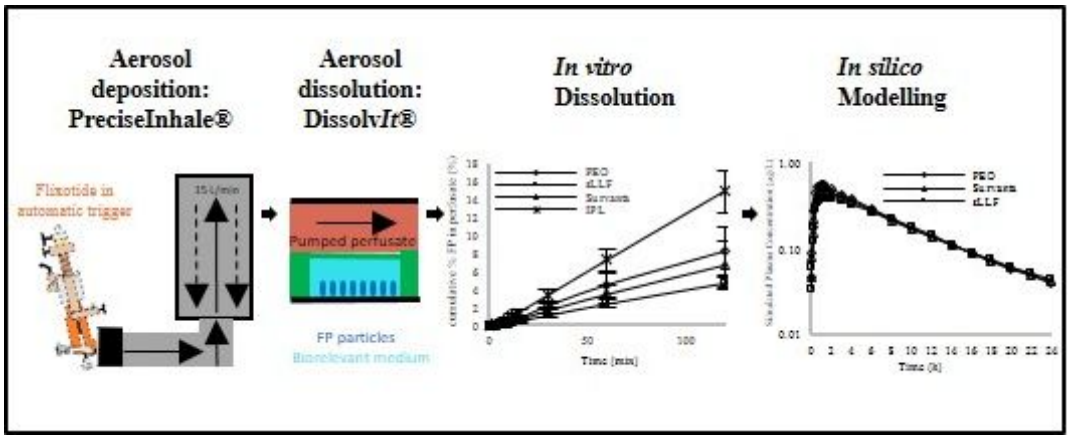
Abstract Graphic



Abstract

The dissolution of inhaled drug particles in the lungs is a challenge to model using biorelevant methods in terms of: (i) collecting a respirable emitted aerosol fraction and dose, (ii) presenting this to a small volume of medium that is representative of lung lining fluid, and (iii) measuring the low concentrations of drug released. We report developments in methodology for each of these steps and utilise mechanistic *in silico* modelling to evaluate the *in vitro* dissolution profiles in the context of plasma concentration-time profiles. The PreciseInhale® aerosol delivery system was used to deliver Flixotide aerosol particles to Dissolv*It*® apparatus for measurement of dissolution. Different media were used in the Dissolv*It* chamber to investigate their effect on dissolution profiles, these were: (i) 1.5% polyethylene oxide with 0.4% L-alphaphosphatidyl choline, (ii) Survanta®, and (iii) a synthetic simulated lung lining fluid (SLF) based on human lung fluid composition. For fluticasone proprionate (FP) quantification, solid phase extraction was used for sample preparation with LC-MS-MS analysis to provide an assay which was fit for purpose with a limit of quantification for FP of 312 pg/mL. FP concentration-time profiles in the flow-past perfusate were similar irrespective of the medium used in the Dissolv*It* chamber (~0.04-0.07%/min), but these were significantly lower than transfer of drug from air-to-perfusate in isolated perfused lungs (0.12%/min). This difference was attributed to the Dissolv*It* system representing slower dissolution in the central region of the lungs (which feature non-sink conditions) compared to the peripheral regions which are represented in the isolated lung preparation. Pharmacokinetic parameters (C_{max}, T_{max} and $AUC_{0-\infty}$) were estimated from the profiles for dissolution in the different lung fluid simulants and were predicted by the simulation within 2-fold of the values reported for inhaled FP (1000 µg dose) administered via Flixotide Evohaler® 250 µg strength inhaler in man. In conclusion, we report methods for performing biorelevant dissolution studies for orally inhaled products and illustrate how they can provide inputs parameters for physiologically based pharmacokinetic (PBPK) modelling of inhaled medicines.

Keywords

Flixotide Evohaler, Fluticasone, PreciseInhale, isolated perfused lungs, simulated lung fluid, Survanta®.

1. Introduction

In vitro dissolution testing is well established for enteral solid dosage forms for quality control purposes, for comparing products under drug classification frameworks and for predicting drug pharmacokinetics *in vivo*[1,2,3,4]. The therapeutic effect of an inhaled particulate aerosol is only realised after drug release into solution, thus investigating the dissolution of solid particle aerosol dosage forms has attracted interest [5-8]. Dissolution testing for orally inhaled products (OIP) is currently a 'hot topic' with research groups adapting a panoply of adaptations of pharmacopoeial apparatus for aerosol collection and dissolution to function as *in vitro* tests for discerning the quality attributes of inhaled medicines. The latest developments in oral biopharmaceutics demonstrate convincingly that biorelevant methods are important if dissolution testing is to be used as an *in vivo* predictive tool and realise its full potential in a regulatory context and to predict clinically-relevant performance [3,4].

The complexity of biorelevant dissolution for inhaled products derives from the need to capture representative aerosol particles in a dispersed manner that reflects their deposition in the lungs, present the particles to low volumes of lung fluid-like dissolution medium and measure reliably the low mass of drug delivered by aerosol medicines. Of the systems reported to date[5-11], none accommodates all these features. The disparate OIP dissolution methods that have been studied tend to be non-integrated and utilise large volumes of dissolution medium, which precludes the use of a dissolution medium that represents human lung lining fluid [12,13]. For some studies of poorly soluble drugs, the medium has been supplemented by addition of protein or phospholipid components, e.g. surfactants such as DPPC [6,14] or lung surfactant preparations such as Survanta® [15]. However, biorelevant media are either expensive or difficult to prepare, and often represent only the surfactant component of distal respiratory tract lining fluid, with the highly abundant proteins absent.

Recently, an integrated apparatus has been developed by Inhalation Sciences for depositing aerosols to a flow past dissolution cell [16], comprising the PreciseInhale® and Dissolv*It*® systems, respectively. The PreciseInhale can deliver carefully controlled doses of aerosols from powder inhalers or pressurised metered dose inhalers to the Dissolv*It* system, in which particle dissolution can be followed by simultaneous observation of aerosol particles using microscopy and measurement of dissolved drug transferred to a flow-past perfusate. Although Dissolv*It* addresses various limitation of dissolution systems used for OIP, the dissolution vessel contains 5.7 µl of a polyethylene oxide (PEO) gel as the dissolution matrix rather than a biorelevant medium. Due to the novelty of the system, there is little reported data on the performance of the system in predicting dissolution[16, 17].

To study clinically-relevant scenarios, dissolution studies to date have focused on the dissolution of poorly soluble inhaled drugs, in particular fluticasone proprionate (FP) [10,11,18]. Delivery of FP to the Dissolv*It* with different biorelevant media in the chamber permits comparison to FP dissolution-absorption profiles in other systems, e.g. isolated perfused lungs (IPL). To perform these experiments requires accurate quantification of sub-micromolar concentrations of FP using a sensitive assay and an efficient extraction method[19,20]. Liquid-chromatography with tandem mass spectrometric detection (LC-MS/MS) provides selective and sensitive analysis of glucocorticoids in biological fluids[21-23]. However, poor repeatability using reported methods[21-23] required development of a new solid phase extraction (SPE) method, which was reliable, quick and required minimal sample preparation and solvent use.

The value of *in vitro* systems is in providing decision-making data, e.g. dissolution measurements for predicting and modelling impacts on drug pharmacokinetics in the early stages of the drug development process. Such data can expedite drug development and prevent unexpected toxico-kinetics and ultimately avoid costly end-stage failures[24]. Reliable predictive models for pharmacokinetics depend on selecting appropriate mathematical

approaches and more current studies tend to utilise *in silico* techniques [25-27]. For modelling dissolution, Backman et al have described how mechanistic models may aid in obtaining a better understanding of dissolution which can be used to predict systemic exposure (AUC) and hence its influence on drug therapeutic effect [28]. For this study, a mechanistic model was developed to evaluate the dissolution data derived from the biorelevant approach using the Dissolv*It* system.

In summary, the aim of the present study was to develop a biorelevant dissolution method by utilising simulated lung fluid in the Dissolv*It* system. To measure the dissolution of FP, a LC-MS/MS method was validated for measurement of low drug concentrations. The effect of dissolution medium on FP aerosol particle dissolution was investigated using three different media: (i) 1.5% polyethylene oxide + 0.4% L-alphaphosphatidyl choline, (ii) Survanta® and (iii) a synthetic simulated lung lining fluid (SLF), synthesised based on human lung fluid composition[29,30]. Finally, an *in-silico* model based on the method of Boger et al[31] was adapted to explore the impact of the dissolution rates derived on pharmacokinetics.

2. Experimental Section

2.1 Materials

Flixotide® 50 µg Evohaler (GSK). Polyethylene oxide (PEO) and L-alphaphosphatidyl choline were supplied by Sigma Aldrich Limited (Dorset, UK) whereas Survanta® was obtained from Abbvie Ltd (Berkshire, UK). The chemicals required for the production of SLF and the preparation of SLF were carried out according to a recently published method[30]. For solid phase extraction validation, the chemicals included were micronized FP (USP grade, purity 98%) supplied by LGM Pharma Inc (Boca Raton, USA), pentadeuterated FP (FP-d5; USP grade, purity 97%) by Insight Biotechnology Limited (Wembley, UK) and rabbit serum, purchased from Sigma-Aldrich Company Limited (Dorset, UK). Chemicals needed for the extraction procedure were zinc sulphate powder, supplied by VWR International Limited (Lutterworth, UK), HPLC-gradient grade acetonitrile, 35% v/v ammonium hydroxide solution and Analytical-Reagent grade dichloromethane, which were all purchased from Fischer Chemical (Loughborough, UK). The materials required for aerosolisation, deposition and dissolution of FP were provided by Inhalation Sciences, Sweden. For FP dissolution in rat IPL, female CD IGS (Sprague Dawley) rats were obtained from Charles River (Sulzfeld, Germany) and the necessary equipment were provided by Inhalation Sciences, Sweden.

2.2 Preparation of calibration curve and validation of assay

Primary stock solutions of FP and FP-d5 were prepared by adding 1 mg of FP or FP-d5 into a 10 mL volumetric flask and filled to the volume with pure acetonitrile, producing 100 µg/mL solutions, and stored at -20°C. A 1 µg/mL FP working solution was prepared by the appropriate dilution of the stock with pure acetonitrile. The calibration standards (156, 313, 625, 1250, 2500, 5000 and 10,000 pg/mL) were prepared from serial dilution of the working solution with pure acetonitrile. Method validation was conducted in terms of linearity, precision (intra-day

and inter-day), accuracy, limit of detection and limit of quantification. Linearity was evaluated by plotting a calibration curve of mean peak area ratio of FP/FP-d5 (n=9) against the concentrations of 7 standards, using a weighted (1/x) linear regression model. The coefficient of variation (%CV) was calculated across 3 calibration sets prepared on the same day for intra-day precision. For inter-day precision, another 3 fresh series of calibration standards prepared on days 2 and 3 were analysed. Accuracy of the data was also evaluated across 9 determinants of each standard, ensuring it was within 15% of each standard concentration. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on Equations. (1) and (2) respectively [19].

$$LOD = 3.3 \times [SD/slope] \quad (1)$$

$$LOQ = 10 \times [SD/slope] \quad (2)$$

Where SD is the standard deviation of the y estimate (peak area ratio) and slope is the gradient of the line.

2.3 Deposition and dissolution of FP aerosol in the Dissolv*It* system

The aerosolisation of Flixotide was carried out by connecting the Flixotide pMDI canister to the US Pharmacopeia Induction Port No 1 (standardised simulation of the throat) of the PreciseInhale aerosol system from Inhalation Sciences (Stockholm, Sweden) (Figure 1). The aerosol particles were deposited on 9 circular microscope glass cover slips, 13 mm in diameter and the dissolution of the deposited particles was investigated by interfacing the particles with the dissolution medium in the Dissolv*It* dissolution system from Inhalation Sciences, (Stockholm, Sweden)[16], thermostatted to 37°C. Pre-warmed dissolution medium, 5.7 µL PEO, Survanta or SLF, was applied to the polycarbonate membrane (pore size 0.03 µm) of each Dissolv*It* dissolution chamber, with the perfusate buffer streaming on the other side. The flow past perfusate consisted of 0.1 M phosphate buffer containing 4% w/v albumin solution,

mixed using a magnetic stirrer. The perfusate was de-gassed using helium to remove excess bubbles and streamed at a flow rate of 0.4 mL/min over a period of 4 h with samples collected by an automated fraction collector at 0, 3, 6, 9, 12, 15, 20, 25, 30, 40, 50, 60, 120 and 240 min.

2.4 Dissolution of FP aerosol in rat isolated perfused lungs

Female rats with body weight 279 ± 20 g, were euthanized with phenobarbital sodium (100 mg/kg, i.p.) and their whole lungs were maintained *ex vivo* as described in other reports [32,33]. The lungs were placed in the artificial thoracic chamber. They were ventilated with room air at 75 breaths/min by creating an alternating negative pressure (-0.2 to -0.8 kPa)^3 inside the chamber, using an Ugo Basile model 7025 animal respirator (Varese, Italy), with a stroke volume of 6 mL, superimposed on a constant vacuum source connected to the chamber. The tracheal air flow velocity and pressure inside the chamber were measured with a heated Hans Rudolph 8430 series pneumotachograph (Kansas City, USA) at 0-3 L/min and a differential pressure transducer from EMKA Technologies (Paris, France), respectively. The physiological lung-function variables: tidal volume (V_t), dynamic lung compliance (C_{dyn})[34] and lung conductance (G_{aw}), which is inversely proportional to lung resistance (RL)[34] were calculated from each breath in real time and logged by a data acquisition system using the EMKA Technologies software IOX v. 6.1a. The lungs were perfused via the pulmonary artery in a single-pass mode, at a constant hydrostatic pressure of approximately 12 cm H_2O and the perfusate reservoir was continually overflowing into a recirculation drain pipe, in order to keep a constant liquid pressure head. Throughout the experiments, the perfusate flow rate after the passage through the lungs (Q_{perf}) was measured gravimetrically using a custom-made fraction collector with a balance. The perfusion medium consisted of Krebs-Henseleit buffer, 5.5 mM glucose, 12.6 mM HEPES and 4% w/v bovine serum albumin. The temperature of the perfusate and the artificial thoracic chamber were maintained at 37°C. The lungs were left to

stabilize for 30 min prior to aerosol exposures and only the lung preparations with stable baseline values for V_t, C_{dyn}, G_{aw} and Q_{perf} during at least a 15-min period were used. The measured values were: V_t: 1.8 ± 0.2 mL, C_{dyn}: 6.6 ± 1.0 mL/kPa; G_{aw}: 279 ± 20 ml/s/kPa, and Q_{perf}: 32 ± 2 mL/min (n=6). Administration of Flixotide aerosol to the IPL was carried out using the PreciseInhale system as described above, where the aerosol was delivered to the lungs by the active dosing system and the system automatically terminated the exposure when the inhaled target dose was reached. The perfusate was sampled using an automatic fraction collector over a 2 h period from the start of the aerosol exposure with sampling intervals of 4.5, 6, 7.5, 9, 12, 15, 30, 60 and 120 min. After the end of the perfusion period, the lungs and trachea were harvested for analysis of the amount of FP retained in the tissues after the perfusion period to enable mass balance calculations. The experiments were approved by a local ethical review board in Stockholm.

2.5 Sample extraction

Samples were prepared for analysis following a new solid phase extraction method. Each sample, 325 µL, was loaded into a deep-well sample plate from Thermo-Scientific (Surrey, UK) followed by 50 µL of internal standard (0.1 µg/mL FP-D5). Zinc sulphate 0.1 M, 300 µL, followed by 75 µL of 10% ammonium hydroxide were added and mixed using a multichannel pipette. The SPE plate was placed on an orbital shaker for 30 min followed by centrifugation at 3700 rpm for 5 min. The samples were then transferred to a pre-conditioned Evolute® Express ABN 10 mg SPE 96-well plate by Biotage (Uppsala, Sweden) and washed by applying low vacuum with 200 µL HPLC-grade water followed by 200 µL of 25% v/v methanol in water. The analytes were eluted twice with 200 µL of pure acetonitrile, once with 100 µL dichloromethane then vacuum centrifuged to dryness. Samples were reconstituted with 30 µL

of 55% v/v acetonitrile in water and sonicated rapidly for 10 min. Finally, an aliquot of the sample (20 µL) was injected into the LC-MS/MS system.

2.6 FP quantification using LC-MS/MS

Quantification of FP was carried out by Waters® Xevo TQ tandem quadrupole mass spectrometer by Waters (Elstree, UK) equipped with an ESI interface, coupled with a Waters Acquity Ultra High Performance LC system (UPLC), equipped with a binary solvent delivery system. Chromatographic separations were carried out on a Waters Acquity UPLC BEH C18 column 130Å, 1.7 µm, 2.1 x 50 mm. The mobile phase was a mix of mobile phase A and mobile phase B, which were 0.1% ammonium hydroxide in water and 1:1 v/v acetonitrile in water, respectively. The flow rate of the mobile phase was 0.2 mL/min with a 2 min gradient from 50% to 95% B. Argon was used as the collision gas and the collision energy was set at 12 V. The LC-MS/MS operations were controlled by the computer software, MassLynx 4.1 and analyte quantification was performed with multiple reaction monitoring using the following transitions: *m/z* 501.4 > 313.1 for FP and *m/z* 506.4 > 313.1 for FP-d5.

2.7 Data analysis.

For the validation process, peak integrations and data analysis were performed using the MassLynx 4.1 computer software. The relationship between peak area ratio and FP concentration (pg/mL) was calculated using the LINEST function in Microsoft Excel. Data was expressed as the mean ± standard deviation of replicate determinations, where $n \geq 3$. For the Dissolv*It* system, the FP transferred to the perfusate was expressed as a percent of the deposited amount on the glass slide. For statistical analysis, One-Way ANOVA was applied to the data followed by Tukey POST-HOC analysis, using the IBM SPSS version 22 software. Data was identified as statistically significant when $p \leq 0.05$.

2.8 Mechanistic modelling

2.8.1. Simulation of plasma concentration-time profiles of fluticasone

A mechanistic physiologically based pharmacokinetic (PBPK) model for predicting the fate of inhaled FP (as illustrated in Figure 2) was developed using Java (Version 1.8.0_111, Oracle, Redwood City, US). The integration of the system of ordinary differential equations was performed via the 8(5,3) Dormand-Prince integrator[35] as realized in the Apache Commons Math library Version 3.6.1 from Apache Software Foundation (Forest Hill, U.S.). The model was adapted from that published by Boger et al.[31]. Briefly, the model was based on the respiratory physiology divided into three compartments; extra-thoracic, tracheobronchial (central lung) and alveolar (peripheral lung) region (Figure 2). The particles deposited in the extra thoracic region were swallowed and transferred to gut, where they were subjected to systemic absorption, based on their bioavailable fraction (F). Particles deposited in the central and peripheral lung regions were modelled for their dissolution in epithelial lung lining fluid, using input from the *in vitro* dissolution experiments in Dissolv*It* system. The *in vitro* data were fitted to a Weibull function to extract the shape and time scale parameters that were then used to model the dissolution of particles in the model. FP permeation in lung tissues and mucociliary clearance of particles deposited in the central lung were modelled as described by Boger et al.[31] The central and peripheral lung areas were perfused by the bronchial blood flow (Q_central lung) and entire cardiac output (Q_cardiac output), respectively. Perfusion-rate limited distribution was assumed to apply for all tissues. System-specific input parameters for central lung, peripheral lung, blood flows and volume of the tissue compartments are provided under supporting information (Tables S1 and S2).

For regional lung deposition modelling, the particle size distribution of the tested formulations was determined using next generation impactor (NGI), resulting in a discrete distribution of

seven particle sizes with corresponding mass fraction deposited (f_0,….f_6). Multiple-Path Particle Dosimetry model MPPD V2.11 2009 from Applied Research Associates Inc (Albuquerque, US) was used to calculate the regional deposition of particles from the tested formulations. A breathing pattern with 2 s inspiration, 1 s expiration, 10 s breath hold and a tidal volume of 625 mL was used[36]. The Yeh-Shum 5-lobe lung model was chosen for the calculations of regional deposition fraction[37]. The drug and formulation specific parameters for FP inhaled in the model are provided under supporting information (Table S3).

2.8.2. Sensitivity analysis of dissolution kinetics

A sensitivity analysis of the pharmacokinetic parameters to the *in vitro* dissolution kinetics of FP was performed using the mechanistic PBPK model (described in section 2.7.1.). Hypothetical *in vitro* dissolution profiles of FP were created by means of numerical approximation with maximum cumulative percent dissolved fixed to mimic the cumulative percent of FP in SLF. The numerical approximations were selected in order to probe three different possible *in vitro* dissolution scenarios: a profile where release greatly exceeded that observed experimentally in SLF (case 1) and two profiles that are similar to SLF but initially more rapid (case 2) or slower (case 3). The data was fitted to a Weibull function to extract the shape (b) and time scale (a) parameters of these profiles. The Weibull equation (Equation 3) was applied to describe the hypothetical dissolution curves and used as an input to the PBPK model. It describes the accumulated fraction of the drug (m) in solution at time t. The location parameter (T_i) is the lag time before the onset of the dissolution, and in all investigated cases was zero.

$$m = 1 - exp\left[\frac{-(t - T_i)^b}{a}\right] \quad (3)$$

3. Results

3.1 Extraction and quantification of fluticasone propionate using LC-MS/MS

As published methods for FP analysis[21-23] proved difficult to replicate with adequate reproducibility and sensitivity, a new SPE method for sample preparation was developed for use with LC-MS/MS for the assay of FP in bio-relevant media. The methodology was easy to perform and the relationship between the mean peak area ratio of FP/FP-d5 and the concentration of FP in the samples was linear (R^2 value=0.999) with inter-day and intra-day precision (CV) being < 20% (in according to ICH guidelines), except for 156 pg/mL. The accuracy for all FP standard concentrations was within 85-115% (Figure 3). The LOD and LOQ were 106 pg/mL and 312 pg/mL respectively. Since the FP concentrations in all dissolution experiments fell within the upper range of the assay, the method was fit for purpose.

3.2 Dissolution of FP in Dissolv*It* and IPL

The penetration of FP, manifested as perfusate concentration, was higher at all time points when the dissolution medium was PEO or Survanta with lipid content lower than that of SLF (Figure 4), in good agreement with the theoretical models. However, overall the influence of medium on FP dissolution was limited since the difference in the FP perfusate concentration values were not statistically significant (One-Way ANOVA, $p>0.05$) between dissolution in any of three lung fluids at most time points, except the difference in FP concentration for PEO and SLF at 20 min. The FP concentration-time profile in perfusate was also similar between PEO and Survanta, both reaching a C_{max} at approximately 20 min. The cumulative percent of FP transferred into the perfusate over time in the Dissolv*It* system showed similar profiles in each dissolution medium reflecting the ranking observed in the perfusate concentrations,

whereas administration to the rat IPL resulted in concentrations of FP and cumulative % of FP in the perfusate that were significantly higher at nearly all time points (Fig 5).

3.3. In silico modelling of FP dissolution.

Pharmacokinetic parameters (C_{max}, T_{max} and $AUC_{0-\infty}$), calculated from the simulated plasma concentration time profiles for the different lung fluid simulants, predicted within two-folds the observed pharmacokinetic parameters of inhaled FP (1000 μg dose) administered via Flixotide Evohaler 250 μg strength inhaler[38] (Figure 6). No significant difference was found between the clinically observed and simulated pharmacokinetic parameters when *in vitro* dissolution input from PEO and Survanta was used in the developed PBPK model. However, differences ($p>0.05$) in C_{max} and $AUC_{0-\infty}$ compared to the clinical data were found when the slower *in vitro* dissolution of FP in SLF was modelled. The $AUC_{0-\infty}$ predicted by the model for all three media were slightly underestimated owing to the underestimation of terminal time points of plasma concentration-time profile of inhaled FP suggesting that FP is retained for longer in the airways, which if incorporated into the model would improve the simulation.

To understand the sensitivity of the predicted PK parameters towards the dissolution profiles of FP, different hypothetical dissolution profiles were created (Figure 7). In the cases where the dissolution-time curves differed from the SLF profile only in terms of faster or slower initial rate (cases two and three), a similar shape parameter described the exponential curves ($b\sim1$). Fitting of an immediate release type hypothetical dissolution profile (case one) resulted in a value describing a sigmoidal curve ($b>>1$). Calculated values of AUC for the cases were similar to the values generated for SLF, which reflecting the fixing of the cumulative percentage of dissolved FP to 9.34% in 4 h. Differences were observed in terms of C_{max} and

T_{max} with profiles when drug dissolution was faster/slower than *in vitro* dissolution profile of FP in SLF. Dissolution profiles mimicking the faster dissolution rates (case one and case two) predicted higher values of C_{max} (6- and 2-fold), and lower values of T_{max} (6- and 4-folds) compared to the values observed in SLF.

4. Discussion

The use of different dissolution media in the Dissolv*It* dissolution assay was investigated. A PEO-based medium is used as the 'standard' solvent for the Dissolv*It* system and possesses a lipid content of 4 mg/mL, which was lower than that of SLF (5.4 mg/mL; Figure 4a). Survanta is a lung surfactant extract concentrate and was diluted (1:5 with water) to normalise the lipid concentration to that of PEO. PEO has no biological relevance beyond providing a viscosity that could be regarded as analogous to that provided by respiratory mucus in the airways[39]. The slower appearance of FP in the perfusate when using SLF compared to PEO or Survanta may reflect slower dissolution or greater retention of FP as a result of the drug preferentially residing or becoming trapped within the more abundant lipid/lamellar structures in SLF, which also contains cholesterol. Cholesterol can form tight nanodomain complexes with DPPC, stabilising DPPC in lipid structures in which FP can be solubilised and retained[40].

Appearance of a low-soluble inhalant in perfusate or plasma is a serial process of dissolution in lung lining fluid followed by diffusion through the air-to-blood barrier. The second step is controlled by barrier thickness and lipid content and distribution within the barrier. While the mathematics of transport in such two-phase heterogeneous barriers was established decades ago[41, 424], the concept was later investigated for lipophilic toxicants in the airway lining

layer[43]. By adding a small amount of surfactant to an aqueous model of the airway lining layer, the penetration of lipophilic benzo(a)pyrene through the experimental barrier was greatly reduced[44]. Thus, a higher content of disperse lipids SLF would be expected to reduce penetration of lipophilic drugs.

Although the simulations in this study were based entirely on human parameters, including the ratio of central:peripheral aerosol deposition, the *ex vivo* rat IPL model was used as a comparator for experimentally-determined dissolution-permeation profiles. The PreciseInhale system provides the advantage of a common delivery platform that can be used to deliver accurate dose and identical respirable aerosol fractions from the pMDI to the *in vitro* dissolution apparatus and *ex vivo* model. The concentration of FP and cumulative proportion of FP in the perfusate was significantly higher at nearly all time points following administration to the rat IPL compared to Dissolv*It*. The higher rate of absorptive clearance was attributed to the IPL possessing a comparatively rapid peripheral (alveolar) dissolution-permeation component in addition to slower central (airway) dissolution-permeation. In contrast, the Dissolv*It* system is hypothesised to model better the dissolution and absorptive clearance mechanisms in the central airways. In the central regions of the lungs, non-sink conditions may be expected as the dose is distributed over a smaller area compared to the alveolar region and dissolved FP molecules are required to diffuse across the 5-20 μm pseudostratified epithelium, compared to 1-2 μm in the alveoli of the lungs, to reach the perfusate[17]. The Dissolv*It*® system possesses an effective dissolution area of 0.95 cm^2 and the penetration distance is approximately 60 μm. Despite being an *ex vivo* non-human model, the IPL is an adaptable tool for teasing out the contributions of dissolution and permeation in different regions of the lungs to drug absorption and local exposure.

As FP exhibits dissolution rate-limited absorption from the lungs of humans[31,45], modelling was carried out to understand the sensitivity of simulated plasma concentration-time profiles of inhaled FP to dissolution profiles. When faster dissolution rates compared to the values observed in SLF were modelled (Figure 7), the higher predicted higher values of C_{max} and lower values of T_{max} were obtained as a result of higher drug concentration in solution during the early stages of the dissolution process. Where the initial rate of *in vitro* dissolution was lower than that in SLF, a lower C_{max} and higher T_{max} value were predicted. This showed clearly the ability of the developed PBPK model to respond to the differences in the *in vitro* dissolution profiles and translate the differences to the respective PK parameters despite the rapid peripheral dissolution and absorption implied by the IPL studies being unaccounted. These results illustrate how dissolution profiles can have significant impact on the PK parameters of a poorly soluble inhaled drug and demonstrate the application of biorelevant *in vitro* assays together with PBPK modelling.

5. Conclusion

We report the development of experimental methods for performing biorelevant dissolution studies for orally inhaled products illustrated by a study into the impact of the dissolution of FP, an archetypal poorly soluble inhaled drug, on plasma pharmacokinetics when the drug was delivered using Flixotide. The *in silico* model was able to translate the *in vitro* data for FP dissolution in the lungs into impacts on physiologically-relevant simulated plasma concentration-time profiles. This approach can lead to enhanced understanding regarding how dissolution processes of inhaled poorly soluble drugs may influence absorptive clearance from the lungs.

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Supporting Information

The following material is available as supporting information:

Table S1	System-specific input parameters for humans
Table S2	System-specific input parameters for the central lung and peripheral lung in humans
Table S3	Drug and formulation specific input parameters for fluticasone propionate
Table S4	Data obtained from FP absorption and concentration profile in the perfusate, following its dissolution in polyethylene oxide in buffer solution (PEO), simulated lung lining fluid (SLF), Survanta and rat isolated perfused lung (IPL). *Difference in parameter is statistically significant (One Way ANOVA, $p < 0.05$). Data expressed as mean ± SD (n=3).

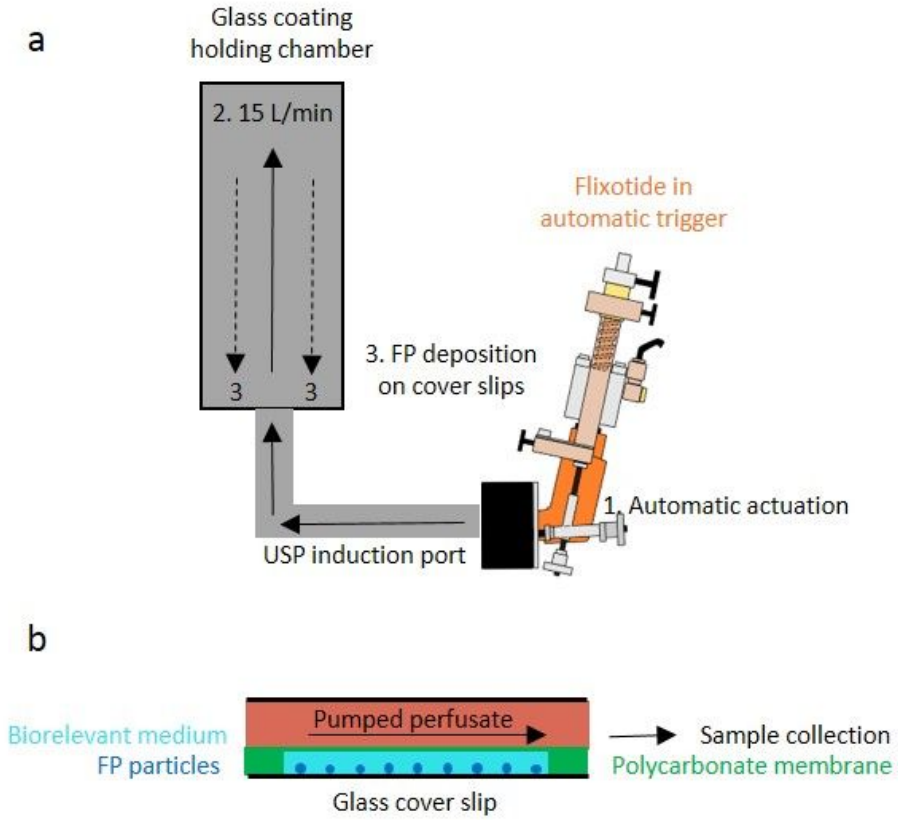


Figure 1. A schematic diagram of a) fluticasone propionate aerosolisation and particle deposition and b) the dissolution system

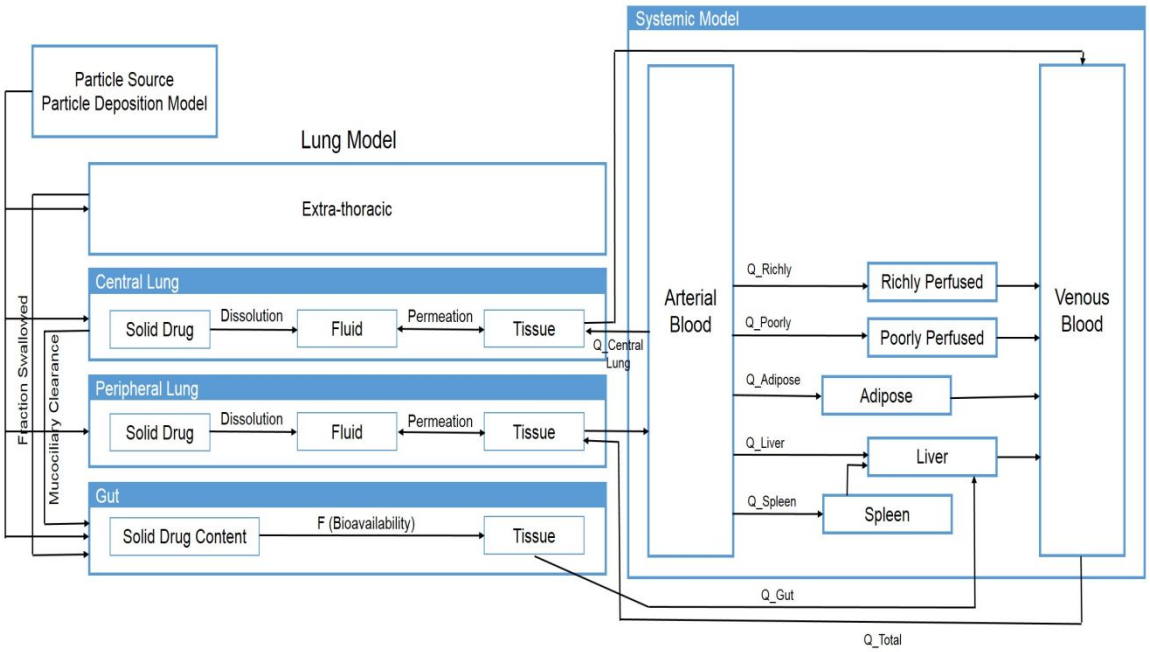
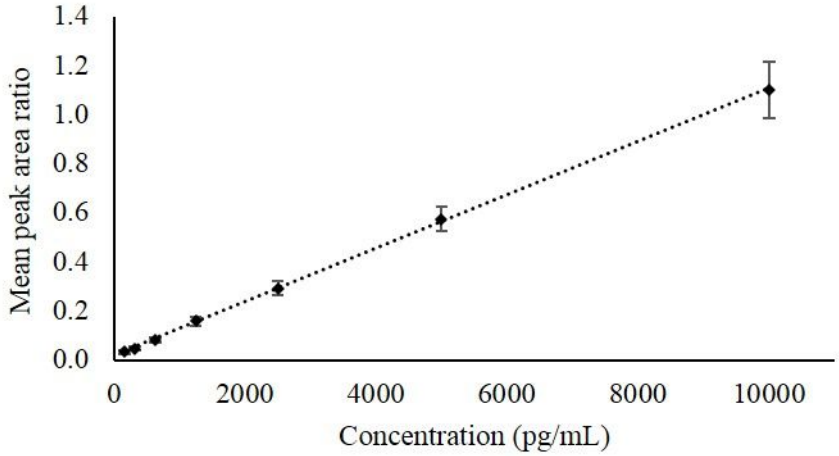


Figure 2. A schematic diagram representing the whole body physiologically based pharmacokinetic (PBPK) model.

a



b

FP (pg/mL)							
Theoretical concentration	156	313	625	1250	2500	5000	10000
Mean measured concentration[a]	142.6 ± 66.5	288.1 ± 57.8	645.6 ± 93.3	1404.1 ± 166.9	2732.2 ± 277.3	5557.4 ± 493.7	10808.5 ± 1110.2
CV (%)	46.6	20.0	14.5	11.9	10.2	8.8	10.3
Accuracy[b] (%)	91.4	92.0	103.3	112.3	109.3	111.1	108.1

[a] n=9

[b] accepted range = 85-115%

Figure 3. Validation of the solid phase extraction and LC-MS/MS assay of fluticasone propionate (FP): a) Linearity of the mean peak area ratio vs concentration; b) FP concentration, precision and accuracy. Data expressed as mean ± SD (n=9).

a

Simulant	Protein concentration (mg/mL)	Lipid concentration (mg/mL)
PEO	-	4.0
SLF	12.9	5.4
Survanta®[a]	0.01-0.16	4.0

[a]Diluted with water to obtain a lipid concentration of 4.0 mg/mL

b

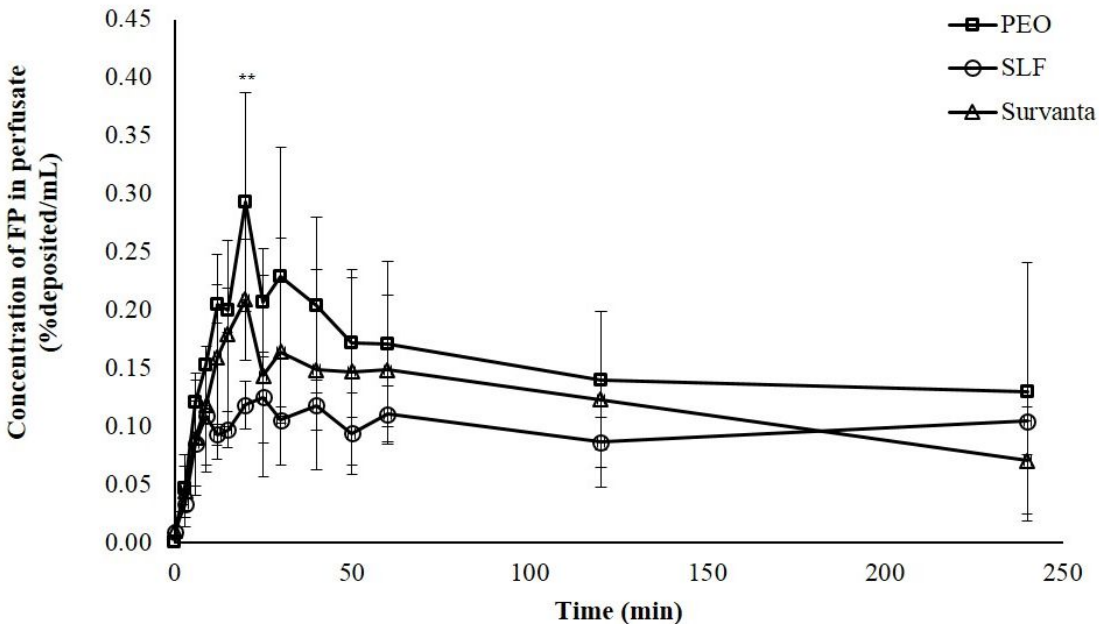


*Figure 4. a) Protein and lipid concentration in polyethylene oxide in phosphate buffer solution (PEO), simulated lung lining fluid (SLF) and Survanta® and b) Concentration of FP in the perfusate over time following dissolution in PEO, SLF and Survanta normalised to mass deposited on the glass cover slips. **Difference in FP concentration in PEO and SLF is statistically significant (One-Way ANOVA, $p<0.05$). Data expressed as mean ± SD (n=3).*

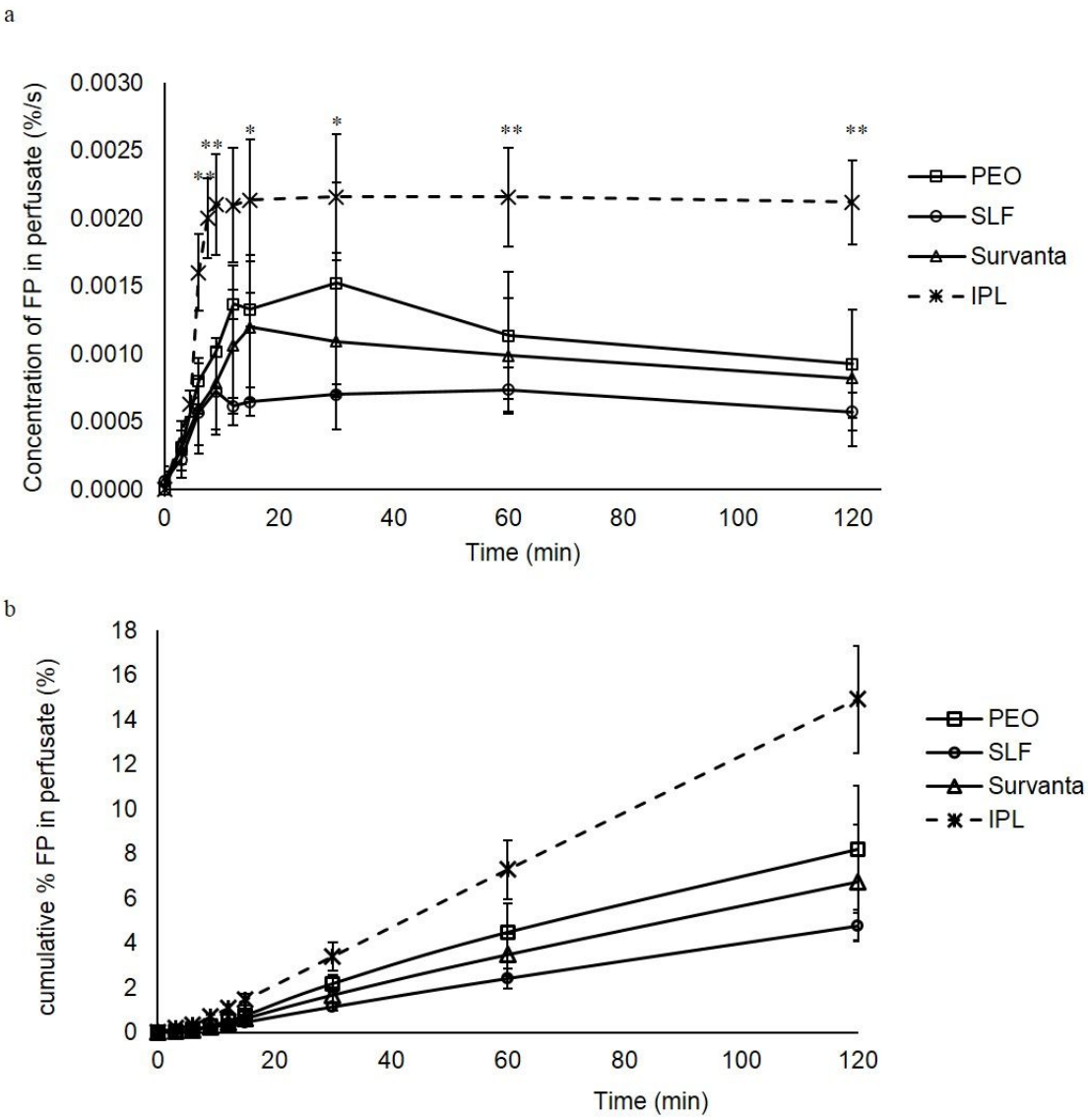
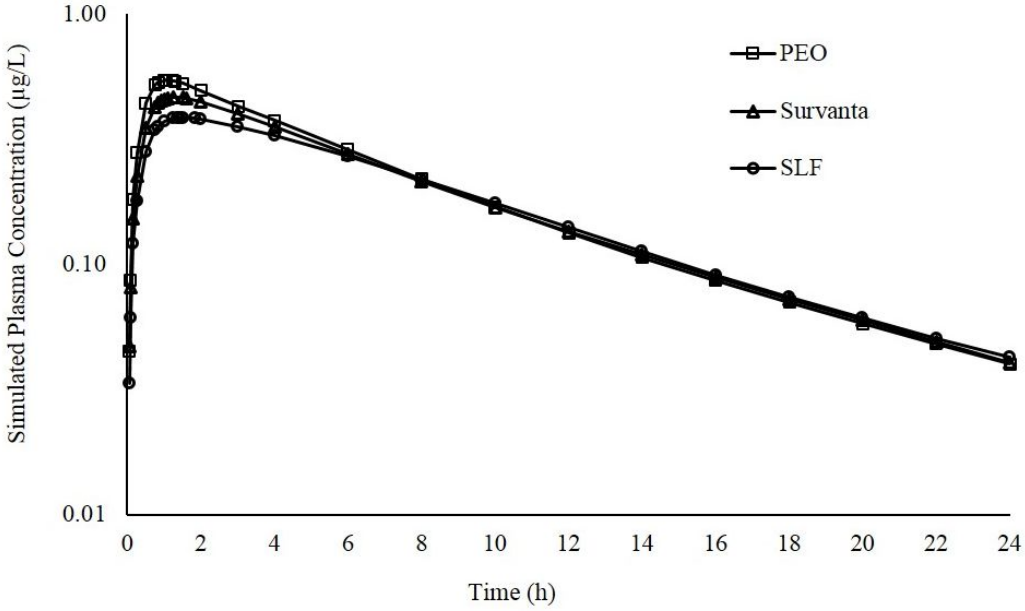


*Figure 5. a) Concentration of FP in the perfusate over time following dissolution in polyethylene oxide in buffer solution (PEO), simulated lung lining fluid (SLF), Survanta and rat isolated perfused lung (IPL). *Difference in FP concentration in IPL and SLF is statistically significant (One Way ANOVA, $p < 0.05$). **Difference in FP concentration in IPL and the remaining three lung fluids, PEO, SLF and Survanta® is statistically significant (One Way ANOVA, $p < 0.05$) and b) Cumulative % of FP transferred into the perfusate over time, following its dissolution in PEO, SLF Survanta and IPL. Data expressed as mean ± SD (n=3).*

a



b

Pharmacokinetic parameter	In-vivo profile*	PEO**	SLF**	Survanta®**
Cmax (µg/L)	0.54	0.54 ± 0.08	0.39 ± 0.03	0.47 ± 0.10
Tmax (h)	0.80	1.11 ± 0.15	1.55 ± 0.26	1.22 ± 0.36
AUC0-∞ (µg/L.h)	6.54	4.97 ± 0.10	4.61 ± 0.09	4.74 ± 0.40

*n=9; reported by Makie et al, 2000.
**n=3

Figure 6. In-silico modelling. a) Simulated plasma concentration of FP over time, following its dissolution in polyethylene oxide in buffer solution (PEO), simulated lung lining fluid (SLF) and Survanta. b) Pharmacokinetic data of FP absorbed in plasma from healthy volunteers, after inhalation of FP pMDI (In-vivo) and of FP absorbed in perfusate, following its dissolution in PEO, SLF and Survanta. Data expressed as mean ± SD (n=3 or 9).

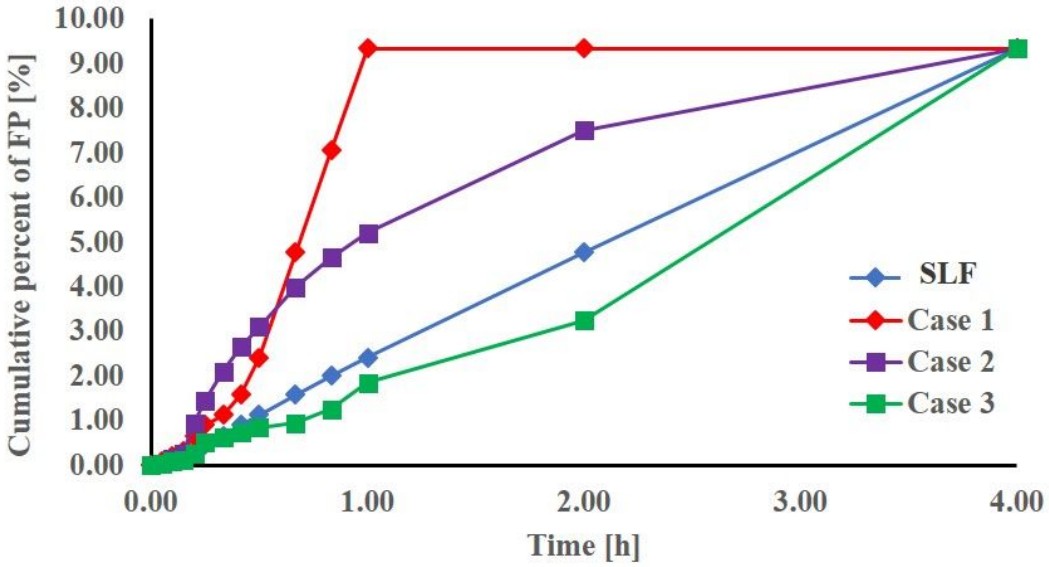


Figure 7. Sensitivity testing using numerical approximation to derive three dissolution profiles that vary from the experimental observations for dissolution of fluticasone in SLF (observed): a profile where release greatly exceeded that observed experimentally in SLF (case 1) and two profiles that are similar to dissolution SLF but initially more rapid (case 2) or slower (case 3).

*Table 1: Fitted Weibull shape factor (b) together with pharmacokinetic data of FP following its dissolution in SLF and artificial dissolution profiles (Cases 1-3); *n=3, **n=1*

Parameter	SLF*	Case 1**	Case 2**	Case 3**
Weibull shape parameter	1.5285 ± 0.08	3.0204	1.1508	1.8716
C_{max} (ug/L)	0.74 ± 0.05	4.61	1.44	0.53
T_{max} (h)	3.01 ± 0.58	0.50	0.75	6.00
$AUC_{0-\infty}$ (ug/L h)	6.46 ± 0.08	6.92	6.87	6.04

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